Journal of Chromatography, 425 (1988) 429–434
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4041

Note

Mitoxantron determination using high-performance liquid chromatography: improved sensitivity by loop-column injection for dual-dose pharmacokinetic studies

MARTIN J. CZEJKA*

Institute for Pharmaceutical Chemistry, Department of Biopharmacy, Währingerstrasse 10, A-1090 Vienna (Austria)

and

APOSTOLOS GEORGOPOULOS

Institute for Chemotherapy, Department of Bacteriology, Lazarettgasse 14, A-1090 Vienna (Austria)

(First received September 24th, 1987; revised manuscript received November 10th, 1987)

The interest that mitoxantron (Fig. 1) presently attracts as a cytostatic in advanced breast cancer, leukaemia and lymphoma therapy [1–3] requires the development of a simple and sensitive assay for pharmacokinetic and bioavailability studies. The drug shows severe side-effects, such as cardiotoxicity [4], therefore proper control of dosage and concentration levels in humans is necessary to reduce toxicity to a minimum. Several high-performance liquid chromatography (HPLC) methods for the determination of mitoxantron in human plasma have been reported [5–9], but they are either too inefficient for routine clinical analysis or they need a very time-consuming extraction step. No method has yet been reported for on-column enrichment of mitoxantron, although this technique is widely used for the rapid extraction of cytostatics and endogenous compounds from different biological matrices [10–13]. In this paper we report the separation and quantification of the compound of interest after rapid extraction from biological material by on-column enrichment followed by isocratic elution of the drug.

0378-4347/88/$03.50

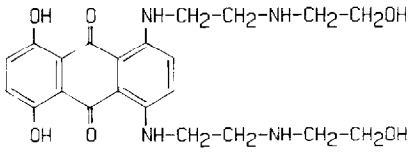

Fig. 1. Structural formula of mitoxantron.

EXPERIMENTAL

Chemicals

Mitoxantron was supplied by Lederle Labs. (Pearl River, NY, U.S.A.). *n*-Heptanesulphonic acid disodium salt, L-ascorbic acid and citric acid were purchased from Merck (Darmstadt, F.R.G., all analytical grade). Deionized and distilled water and methanol (LiChrosolv, Merck) were used for extraction and mobile phase preparation.

HPLC equipment

The liquid chromatograph consisted of a 420 pump (Kontron, Vienna, Austria), a LiChrosorb RP-8 guard column (10 μm, 10×4.2 mm I.D.) and a LiChrosorb RP-8 analytical column (5 μm, 150×4.2 mm I.D.). Columns were connected by an "eco-tube" cartridge system (Bischoff, Leonberg, F.R.G.), and were thermostatted by a column oven 830 (Kontron). Detection was performed by an Uvikon 430 photometer connected to an Anacomp 220 integration unit (both Kontron). Injection was performed with a Rheodyne 7125 valve with a loop-column (Spherisorb ODS, 30 μm, 15×4.2 mm I.D.) instead of a loop.

Chromatographic conditions

The mobile phase was methanol–water (40:70, v/v), containing 5 mmol *n*-heptanesulphonic acid disodium salt. The HPLC system was operated at 1.0 ml/min (pressure 100 bar) and thermostatted at 30 °C. Detection was performed by measuring the absorbance at 656 nm and a sensitivity of 0.005, with a response time of 2 s.

Sample handling

Plasma samples (1.0 ml) were obtained by venepuncture at various time intervals and stabilized by immediate addition of 0.1 ml of 3% (w/v) ascorbic acid in 0.1 *M* citrate buffer (pH 3.0). Samples were stored at −70 °C until analysis.

Extraction procedure

Plasma samples were centrifuged for 3 min at 4000 *g* prior to injection. The Rheodyne valve was set to the "load" position, and the loop column was flushed with 1.0 ml of *n*-heptanesulphonic acid solution (0.5% in distilled water), followed by injection of sample (0.1–0.5 ml). Then 1.5 ml of *n*-heptanesulphonic acid solution was injected into loop-column to remove matrix constituents. Finally the valve was switched to the "inject" position for chromatography.

RESULTS AND DISCUSSION

Extraction procedure

Various sample clean-up procedures and isocratic HPLC assays have been reported for mitoxantron. These methods either have time-consuming extraction steps [5,6] or limited sensitivity [5] or require plasma volumes of up to 10 ml to gain sufficient sensitivity [7]. The advantage of the proposed extraction method is based on rapid sample clean-up (within 25 s) and high accuracy. There is no decomposition of the drug and no loss of sample during isolation from the matrix. Moreover, instead of dilution, the sample is preconcentrated by direct injection on the loop-column. Isolation of the drug from the matrix requires only three steps: injection of wash fluid for polar equilibration of the loop-column, injection of sample, and finally injection of wash fluid to remove matrix constituents. The method offers high sensitivity by injection of plasma volumes up to 1 ml and can be adapted for automatic HPLC determination of the drug in routine clinical analysis. The loop-column could be used for ca. 80 injections of 0.2 ml of plasma before renewal. The need for this change was indicated by an increase of back-pressure to 200 bar: the standard working pressure was 90–100 bar.

Chromatographic isolation

Acetonitrile was the most suitable organic modifier for the separation of mitoxantron. However, in the proposed method, designed for routine analysis of mitoxantron in biological samples, methanol was preferred as modifier because of its lower toxicity. The chromatograms in Fig. 2 show that mitoxantron in plasma extracts was completely separated from endogenous compounds using a mobile phase containing 36% methanol. Mitoxantron elutes at 8 min, and its two possible metabolites elute between 4 and 7 min. The parent drug is the only compound of interest in pharmacological and pharmacokinetic investigations, because the metabolites play only a minor role in such studies.

Photometric detection

On-column enrichment makes possible photometric measurement of the drug down to the low nanogram level. The detection sensitivity as well as the high detection selectivity of the present method were obtained by photometric measurement of the eluate at 656 nm. The detection limit was 2 ng/ml by injection of 2 ml of plasma (signal-to-noise ratio 5:1).

Quantitative determination

Quantitation was effected by the external standard method, comparing the peak heights or peak areas of a calibration curve with data obtained from sample chromatograms. The calibration curve was linear in the range 10–900 ng for peak heights ($y=2.77x+0.09$, $r=0.979$, $n=5$) where y is the peak height in mV and x is the drug concentration (ng/ml) and from 50–1360 ng for peak areas ($y=0.13x-0.07$, $r=0.996$, $n=9$) where y is the peak area in V min and x is the drug concentration in ng/ml.

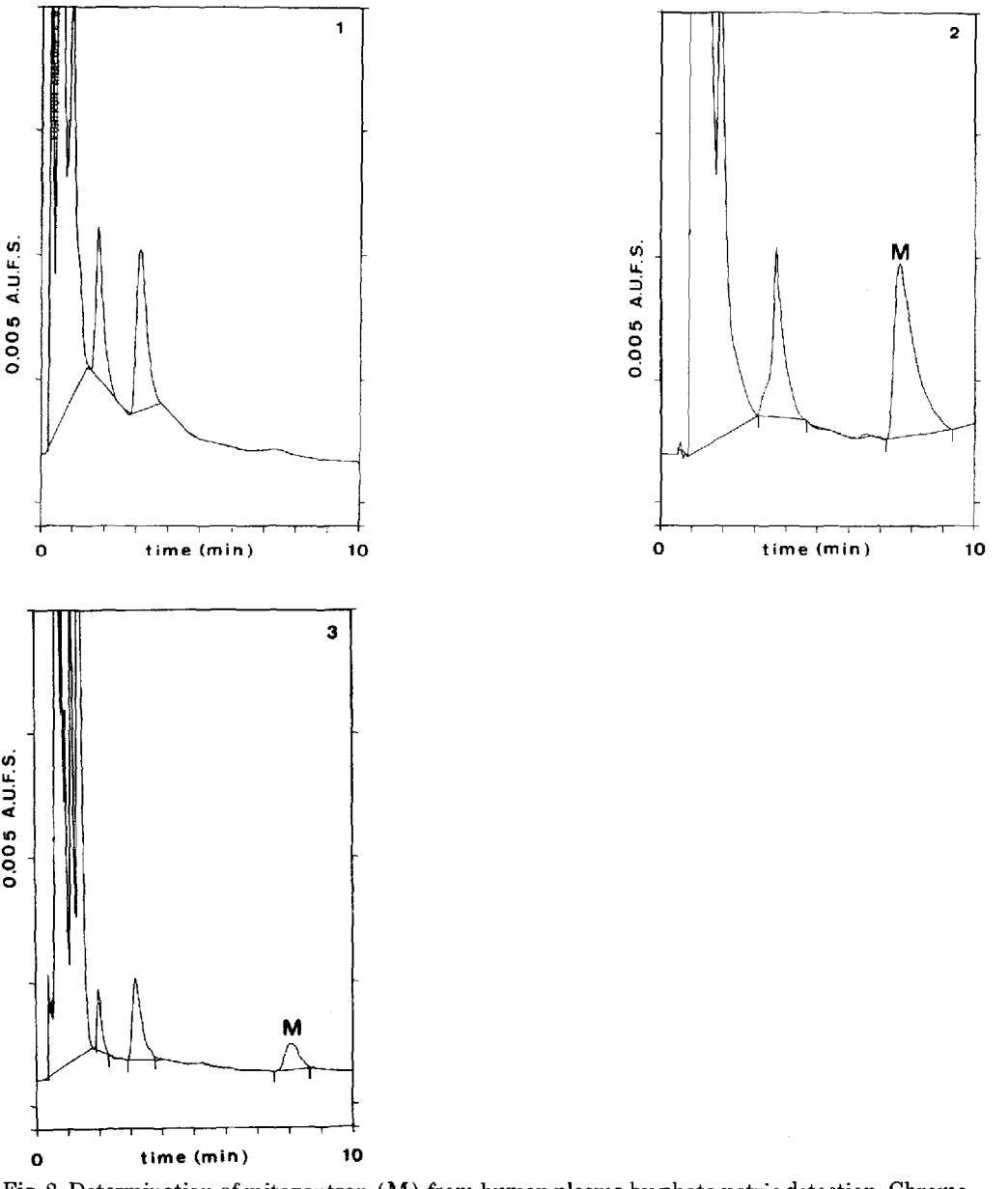

Fig. 2. Determination of mitoxantron (M) from human plasma by photometric detection. Chromatograms from blank plasma (1) and plasma samples, containing 950 ng/ml (2) and 180 ng/ml (3). For chromatographic conditions see text.

Precision of assay

Analysis of fifteen standard curves over 3 months indicated that the day-to-day coefficient of variation (C.V.) was 1.8% and the within-day C.V. was 1.3% (concentration range 50–800 ng/ml).

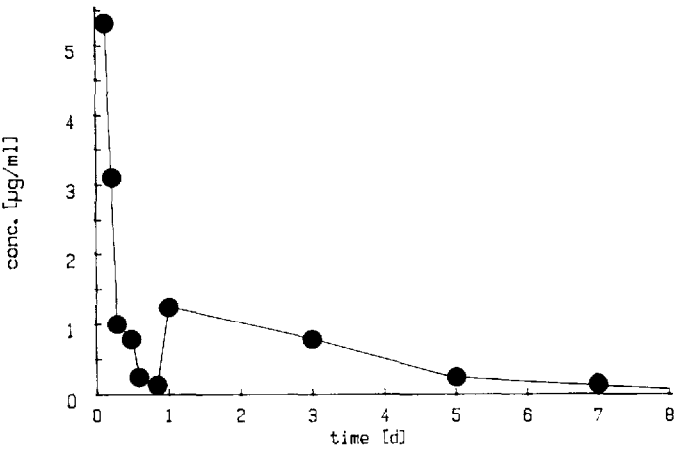

Fig. 3. Plasma concentration–time curve of a mitoxantron-treated patient after a dual dose (for pharmacokinetic parameters see Table I).

TABLE I

MITOXANTRON PHARMACOKINETIC PARAMETERS AFTER A DUAL DOSE IN A MITOXANTRON-TREATED FEMALE

Parameter	First dose	Second dose
Dose (mg/kg body weight)	0.3	0.1
Hypothetical initial dose (µg/ml)	6.6	1.7
Elimination half-life (h)	2.7	41.1
Volume of distribution at steady state (l)	2.9	3.8
Partition coefficient (ml/g)	0.05	0.06

Clinical application

An initial dose of 0.3 mg/kg body weight was given by chemobilization followed by a second dose of 0.1 mg/kg after 16 h. Plasma levels of mitoxantron-treated patients were analysed over a period of 2 weeks, Fig. 3 shows a typical plasma concentration–time curve after such a dual dose; the corresponding pharmacokinetic variables are listed in Table I.

CONCLUSION

The proposed method provides excellent sensitivity and accuracy for rapid loop-column extraction and HPLC analysis of mitoxantron in human plasma. The analytical assay was used to monitor plasma levels in eight mitoxantron-treated patients.

ACKNOWLEDGEMENT

We thank Ing. M. Nösterer from Kontron Austria for supply of the Anacomp 220 controller and integration unit.

REFERENCES

1 P.A. Paciucci, T. Ohnuma, J. Cuttner, R.T. Silver and J.F. Holland, Cancer Res., 42 (1983) 3919.
2 R.C. Stuart-Harris, T. Bozer, N.A. Pavlidis and I.E. Smith, Cancer Chemother. Pharmacol., 12 (1984) 1.
3 C.A. Coltman, T.M. McDaniel, S.P. Balcerzak, F.S. Morrison and D.D. van Hoff, Invest. New Drugs, 1 (1983) 65.
4 E. Bachmann, E. Weber and G. Zbinden, Cancer Treat. Rep., 71 (1987) 361.
5 D.L. Reynolds, L.A. Sternson and A.J. Repta, J. Chromatogr., 222 (1981) 225.
6 G. Ehninger, B. Proksch and E. Schiller, J. Chromatogr., 342 (1985) 119.
7 G. Ehninger, B. Proksch, G. Heinzel, E. Schiller, K.H. Weible and D.L. Woodward, Invest. New Drugs, 3 (1985) 109.
8 Y.M. Peng, D. Ormberg, D.S. Alberts and T.P. Davis, J. Chromatogr., 233 (1982) 235.
9 S.J.P. Van Belle, T.J. Schoemaker, S.L. Verwey, A.C.A. Paalman and J.G. McVie, J. Chromatogr., 337 (1985) 73.
10 C.A. Riley, W.R. Crom and W.E. Evans, Ther. Drug Monit., 7 (1985) 455.
11 P.J. Arnold, R. Guserle, M. Wörz and V. Luckow, Chromatographia, 22 (1986) 431.
12 G. Guinn and D.L. Hendrix, J. Chromatogr., 348 (1985) 123.
13 C. Wegner, M. Trotz and H. Nau, J. Chromatogr., 378 (1986) 55.